REMARKS

1. Description

1.1. The Examiner has taken the position that equations (a)-(c), as stated in claim 93, are inconsistent with the disclosure.

We agree with the Examiner vis-a-vis replacing "NAD" with --NAD^+-- (in reaction (b)), and "NH3" with --NH_4^+-- in (a) and (c). Claim 93 has been so amended.

However, the Examiner is incorrect regarding the change from NAD to $NADP^+$ and from NADH to NADPH in reaction (a). The Examiner refers to page 17 in connection with this rejection. The reaction set out in line 4 on page 17 is not the reaction referred to under paragraph (a) of claim 93. Rather, that is the reaction catalysed by the NADPH dependent glutamate dehydrogenase encoded by *GDH1*. That is the enzyme which is reduced or eliminated according to paragraph (ii) of claim 93. The invention in this aspect lies in replacing that enzyme activity with the enzyme activity of the enzyme GDH2 which uses NADH rather than NADPH. This is explained at page 17, lines 14 onwards: "it has been demonstrated as described herein below and in Example 1, that formation of glycerol can be reduced by deleting *GDH1* and over expressing *GDH2*". Page 17 lines 6 onwards explains that the enzyme encoded by *GDH2* catalyses the opposite reaction as that of GADH1 but under the formation of NADH. These enzyme reactions are of course reversible and the effect of deleting or reducing activity of GDH1 is that GDH2 catalyses the NADH consuming reaction set out in paragraph (a) of the claim as described commencing at line 16 of page 16 "these manipulations result in a genetically modified yeast strain synthesizing glutamate under consumption of NADH rather than NADPH".

This replacement of GDH1 with GDH2, its NADH-dependent isoenzyme is described in detail in connection with Example commencing on page 63.

That GDH2 catalyses the reaction in the direction specified in paragraph (a) of the claim is made explicit at page 68, line

26 reading "as described earlier glutamate can be regenerated from 2-oxoglutamate by either of the two isoenzymes of glutamate hydrogenase, encoded by *GDH1* and *GDH2*, under consumption of NADPH or NADH respectively".

1.2. The Examiner also questions whether there is "description" for a microbial cell "of any origin", and for "any enzyme that might be able to catalyze the recited reactions".

In general, the written description requirement forbids attempts to reconceptualize the invention in a manner not set forth in the original disclosure and claims. While closely intertwined with the enablement requirement, it should not be confused with the latter. We believe that the Examiner may have confused the two.

In general, the original claims of the application necessarily satisfy the written description requirement. See, e.g., In re Koller, 204 USPQ 702 (CCPA 1980), which has never been overruled.

The PTO says that Eli Lilly identified a special set of circumstances in which the words of the claim were effectively empty of meaning, because they just were reciting a name, without any structural information, for a novel chemical compound. The PTO concluded that "in most cases", an originally filed claim is its own written description. See preamble to the January 5, 2001 written description guidelines.

In this case, the original claims recited

"microbial cells" (claim 1)
"fungal cell" (claim 6)
"yeast cell" (claim 6)
"bacterial cell" (claim 6)

We are not aware of any case that has applied the Eli Lilly doctrine outside of the context of a biomolecule (especially DNA) defined by its sequence. There is no justification for saying that a kind of cell cannot be contemplated without disclosure of (unspecified) structural information about the cell.

Nonetheless, purely because the application is under final rejection, and without prejudice or disclaimer, we have amended claim 93 to specify that the microbial cells are fungal cells. Please note that we believe that yeast cells are a kind of fungal cell, so that claims 113-116 remain properly dependent on 93. If the Examiner disagrees, we will need to amend 93 to explicitly recite yeast cells.

We turn next to the issue of enzymes from nonfungal sources. Although the description contains details only of work conducted on yeast, the biochemical pathways with which the invention is concerned are extremely fundamental ones which are common to most life forms. It is therefore a reasonable expectation that enzymes which are functionally equivalent to those described in the specification exist and regulate the same pathways universally. Furthermore, because the substrates for the enzymes are common chemicals fundamental to ammonia assimilation as a nitrogen source and because the enzymes make use of cofactors which are universal (NAD, NADH) and the universal energy source ATP/ADP, there can be no reasonable expectation that an enzyme which is functional in one species will not be functional in the same way in another.

Most organisms possess glycolysis, the pentose phosphate pathway or the citric acid pathway. Glycolysis occurs in almost every living cell and many kinds of organisms possess the pentose phosphate pathway and the citric acid cycle plays a central role in eukaryotes as well as most prokaryotes. From these pathways all of the mentioned first and second metabolites discussed in the specification are derived. For example, lactic acid is derived from pyruvate, the end product of glycolysis, via a reaction catalysed by lactate dehydrogenase. Ethanol can also be derived from pyruvate for example via a two step pathway using the reactions that are catalysed by pyruvate decarboxylase and alcohol dehydrogenase. Terpenes and sterols are derived from acetyl-CoA an intermediate of for example in the citric acid cycle. There are many more examples. All of the mentioned

pathways are redox dependent, and a change in ammonia assimilation will hence influence the production of these compounds as described in the application.

The invention of the present patent application is to apply genetic modifications to ammonia assimilation. Ammonia assimilation is highly conserved in all organisms. The introduction of inorganic nitrogen (ammonia, nitrate, nitrogen (N2, etc.) into organic compounds proceeds always via ammonia. Attached is a drawing (Figure A) illustrating this. Nitrate, and nitrite and molecular nitrogen will always first be reduced to ammonia and only then further be metabolised into organic compounds. As can be seen from Figure A the first upper pathways describe ammonia assimilation via the GS-GOGAT pathway and glutamate dehydrogenase. It is those pathways that are subject to genetic modifications and to improvement of production of a first metabolite and to reduction of the production of a second metabolite. Hence, genetic modification in ammonia assimilation in different organisms will lead to change in the production of all relevant first and second metabolites.

Accordingly, it is certainly to be expected that the invention would be workable in different fungi cells in the same manner as described in the application.

Please find attached a print of the text of a web page of Purdue University which discusses the near universality of the two isoforms of glutamate dehydrogenase (GDH). This clearly shows that in fungi, including yeast, both GDH forms are found as a matter of generality and have been previously identified in the published art. It is also revealed that the two forms of GDH are to be found in a number of different bacteria and also in plants (Arabidopsis and Zea).

In addition, we enclose abstracts of various papers as discussed below.

Dantzig et al demonstrates the existence of the NADH-dependent glutamate hydrogenase in *Neurospora* (a fungus). Mtolera discloses the existence of the enzyme in the red alga

Gratilaria sordia.

Storey et al demonstrates the existence of the enzyme in *Osteoglossum* and *Arapaima* (fish).

Dennen et al demonstrates its existence in *Schizophyllum* (a mushroom type fungus).

Lé John et al discloses the existence of the two isoenzyme GDH's in the bacterium *Thioacillus*.

The NADH dependent glutamate synthase GLT1 is equally universal. Enclosed is a web page from the European Bio Informatics Institute disclosing that it is a key enzyme in the early stages of assimilation of ammonia in bacteria, yeasts and plants. Enclosed also is an abstract by Ishiyama et al disclosing that it is present in *Oryza sativa seedlings* and in the root modules of alfalfa where it derives from a nitrogen fixing bacterium (see Vanoni et al.).

The enzyme GLN1 is equally universal. Enclosed is a copy of another web page from Purdue University regarding this and demonstrating the universality of this protein in fungi and yeasts generally.

Based upon these materials it is reasonable to conclude that the written description of the invention in the application would readily be understood by a skilled reader to be a fair exemplification of the invention as it applies across the spectrum of fungi including yeast and the skilled reader would entertain no doubt that the inventors were in full possession of the invention as claimed at the relevant date.

2. Enablement/Deposit

Claims 102, 105, 108, 111 and 115-116 refer to specific deposited biological matters, and the Examiner requires compliance with 37 CFR 1.801-1.809.

Since the specification discloses the depository accession numbers (DSM 12267, DSM 12274, DSM 12275, DSM 12276 and DSM 12277), the deposits quite obviously were made before the filing date. Hence, we don't need a "chain-of-custody" statement.

In addition, DSM is a Budapest Treaty depository, and the deposits were made under that Treaty.

Hence, we need only (and do) aver that the deposited material was accepted for deposit under the Budapest Treaty, and that all restrictions on the availability to the public of the material so deposited will be irrevocably removed upon the granting of the patent.

The specification has been amended to set forth the full name and address of the depository and the date of each deposit.

Respectfully submitted,

BROWDY AND NEIMARK, P.L.L.C.
Attorneys for Applicant

By: _____
Iver P. Cooper
Reg. No. 28,005

Enclosures
-Dantzig et al. (abstract)
-Storey et al. (abstract)
-Dennen et al. (abstract)
-LeJohn et al. (abstract)
-EBI (web page)
-Ishiyama et al. (abstract)
-Vanoni et al. (abstract)
-Purdue (two web pages)
-Figure A
-Trepp et al. (abstract)
-Mtolera (abstract)

624 Ninth Street, N.W.
Washington, D.C. 20001
Telephone: (202) 628-5197
Facsimile: (202) 737-3528
IPC:lms
G:\ipc\u-z\WHBE\Nielsen4\PTO AMENDAFTFNL.wpd

JOURNAL OF BACTERIOLOGY, Mar. 1979, p. 1333-1339
0021-9193/79/03-1333/07$02.00/0

Vol. 137, No. 3

Regulation of Glutamate Dehydrogenases in *nit-2* and *am* Mutants of *Neurospora crassa*

ANNE H. DANTZIG,†* FRANCIS L. WIEGMANN, JR., AND ALVIN NASON‡

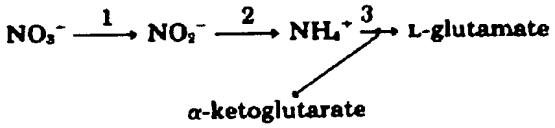
McCollum-Pratt Institute, The Johns Hopkins University, Baltimore, Maryland 21218

Received for publication 27 December 1978

The regulation of the glutamate dehydrogenases was investigated in wild-type *Neurospora crassa* and two classes of mutants altered in the assimilation of inorganic nitrogen, as either nitrate or ammonium. In the wild-type strain, a high nutrient carbon concentration increased the activity of reduced nicotinamide adenine dinucleotide phosphate (NADPH)-glutamate dehydrogenase and decreased the activity of reduced nicotinamide adenine dinucleotide (NADH)-glutamate dehydrogenase. A high nutrient nitrogen concentration had the opposite effect, increasing NADH-glutamate dehydrogenase and decreasing NADPH-glutamate dehydrogenase. The *nit-2* mutants, defective in many nitrogen-utilizing enzymes and transport systems, exhibited low enzyme activities after growth on a high sucrose concentration: NADPH-glutamate dehydrogenase activity was reduced 4-fold on NH_4Cl medium, and NADH-glutamate dehydrogenase, 20-fold on urea medium. Unlike the other affected enzymes of *nit-2*, which are present only in basal levels, the NADH-glutamate dehydrogenase activity was found to be moderately enhanced when cells were grown on a low carbon concentration. This finding suggests that the control of this enzyme in *nit-2* is hypersensitive to catabolite repression. The *am* mutants, which lack NADPH-glutamate dehydrogenase activity, possessed basal levels of NADH-glutamate dehydrogenase activity after growth on urea or L-aspartic acid media, like the wild-type strain, and possessed moderate levels (although three- to fourfold lower than the wild-type strain) on L-asparagine medium or L-aspartic acid medium containing NH_4Cl. These regulatory patterns are identical to those of the *nit-2* mutants. Thus, the two classes of mutants exhibit a common defect in NADH-glutamate dehydrogenase regulation. Double mutants of *nit-2* and *am* had lower NADH-glutamate dehydrogenase activities than either parent. A carbon metabolite is proposed to be the repressor of NADH-glutamate dehydrogenase in *N. crassa*.

Many eucaryotic microorganisms possess two distinct glutamate dehydrogenases for the interconversion of glutamate with ammonium and α-ketoglutarate, a key step in nitrogen metabolism (14). Evaluation of the factors important in the regulation of the glutamate dehydrogenases has proved difficult since the two enzymes are regulated concurrently but oppositely by both carbon and nitrogen nutrients in the medium; i.e., when one activity is high, the other is low (19, 30–33). This paper examines the regulation of the glutamate dehydrogenases in *Neurospora crassa*.

The nitrate assimilatory pathway converts inorganic nitrogen as nitrate to L-glutamate as follows:



The first two steps are catalyzed, respectively, by nitrate reductase (12, 13, 25, 26) and nitrite reductase (5, 23), and the third, by the glutamate dehydrogenases. NADPH-glutamate dehydrogenase is believed to convert NH_4^+ and α-ketoglutarate to L-glutamate, whereas NADH-glutamate dehydrogenase degrades L-glutamate (30). Each enzyme has been purified to homogeneity (3, 39).

Most mutants of the pathway have been isolated by three selection procedures: lack of growth on nitrate as a sole nitrogen source has yielded the mutants *nit-1*, *nit-2*, and *nit-3* (36); lack of growth on ammonium, the *am* mutants

† Present address: Department of Human Genetics, Yale University School of Medicine, New Haven, CT 06510.
‡ Deceased.

http://www.pubmedcentral.nih.gov/picrender.fcgi?artid=218317&action=stream&blobtyp... 17/09/2004

Storey, K.B., Guderley, H.E., Guppy, M. and Hochachka, P.W. 1978. Control of ammoniagenesis in the kidney of water- and air-breathing osteoglossids: control of glutamate dehydrogenase. *Can. J. Zool.* 56, 845-851.

Control of ammoniagenesis in the kidney of water- and air-breathing osteoglossids: characterization of glutamate dehydrogenase

K. B. STOREY, H. E. GUDERLEY, M. GUPPY AND P. W. HOCHACHKA

Abstract

Glutamate dehydrogenases (EC 1.4.1.2) from the kidney of *Osteoglossum bicirrhosum* (called aruana) and *Arapaima gigas* were kinetically characterized. The two enzymes exhibited several common characteristics including Vmax activity ratio, pH optimum, affinity for cofactors, a marked preference for NAD(H) over NADP(H), and a very low affinity for NH4+. A variety of regulatory metabolites affected both enzymes. GTP and GDP were inhibitory while ADP, ATP, AMP, and leucine activated the enzymes. Both enzymes displayed potent product inhibition which was partially reversed by low levels of ADP. *Arapaima* kidney glutamate dehydrogenase was tightly regulated by the adenylate and guanylate nucleotides, inhibition by GTP and GDP and deinhibition by ADP and AMP being much stronger for this enzyme than for the aruana enzyme. Aruana glutamate dehydrogenase, however, was more responsive to NAD-NADH control. The enzyme was more sensitive to NAD(H) product inhibition and this inhibition was poorly reversed by ADP. From these data, it was concluded that both fish kidney glutamate dehydrogenases could function in glutamate oxidation in vivo. However, the *Arapaima* enzyme appeared most clearly adapted to a catabolic roles activity being more tightly linked to the energy status of the mitochondrion. Conversely, the aruana enzyme displayed regulatory properties allowing it the potential to function in NADH oxidation during periods of hypoxic stress.

JOURNAL OF BACTERIOLOGY, Mar., 1967, p. 904-913
Copyright © 1967 American Society for Microbiology
Vol. 93, No. 3
Printed in U.S.A.

Regulation of Glutamate Dehydrogenases During Morphogenesis of *Schizophyllum commune*

DAVID W. DENNEN[1] AND DONALD J. NIEDERPRUEM
Department of Microbiology, Indiana University Medical Center Indianapolis, Indiana

Received for publication 22 October 1966

The specific activities of two glutamate dehydrogenases (GDH), one requiring nicotinamide adenine dinucleotide (NAD) and the other specific for nicotinamide adenine dinucleotide phosphate (NADP), varied during growth of *Schizophyllum commune* as a function of the stage of the life cycle and the exogenous nitrogen source. During basidiospore germination on either glucose-NH_3 or glucose-glutamate medium, NADP-GDH increased six- to eightfold in specific activity, whereas NAD-GDH was depressed. During dikaryotic mycelial growth on either nitrogen source, the two GDH increased in a 1:1 ratio, whereas, during homokaryotic mycelial growth on glucose-NH_3, NADP-GDH activity was depressed and NAD-GDH increased six- to eightfold. Homokaryotic mycelium cultured on glucose-glutamate medium yielded high NADP-GDH activities and normal NAD-GDH activities. Intracellular NH_3 concentration and NADP-GDH activities were inversely related during spore germination and homokaryotic mycelium growth, whereas guanosine-5'-triphosphate (GTP) and L-glutamine specifically inhibited NAD- and NADP-GDH respectively in vitro. GTP inhibition was shown in extracts from cells at all stages of the life cycle. Basidiospore germling extracts contained an NADP-GDH essentially resistant to L-glutamine inhibition.

The influence of either NH_3 or L-glutamate as sole nitrogen source on the specific activity of nicotinamide adenine dinucleotide (NAD)- and NAD phosphate (NADP)-glutamate dehydrogenases (GDH) in fungi has been called coordinate regulation (15), a term which implies the simultaneous action of these metabolites or their derivatives as inducers for one enzyme and corepressors for the other. L-Glutamate has been described as a corepressor of NADP-GDH in *Neurospora crassa* (1) and *Piricularia oryzae* (8), and NH_3 has been suggested as corepressor of NAD-GDH in *Saccharomyces cerevisiae* (7). In addition, urea, one of the products of glutamate metabolism, appears to act simultaneously to induce NAD-GDH and to repress NADP-GDH in *N. crassa* (15). The repression of NAD-GDH and the induction of NADP-GDH by NH_3 in these organisms is in agreement with their cellular function documented by studies on the *am* mutants of *N. crassa* (4). In this system, NADP-GDH is anabolic (i.e., responsible for the formation of glutamate from α-ketoglutarate and NH_3), whereas NAD-GDH is catabolic and catalyzes the reverse reaction.

[1] Present address: Eli Lilly and Co., Indianapolis, Ind.

The finding that NADP-GDH activity in *Schizophyllum commune* is depressed during growth of vegetative mycelium on glucose-NH_3, the reverse of that on glucose-glutamate medium, suggested that the GDH of this basidiomycete are regulated differently than in other fungal systems (3). Furthermore, the specific activities of these enzymes also changed with morphogenesis of this mushroom (D. W. Dennen and D. J. Niederpruem, Bacteriol. Proc., p. 58, 1966). The present report assesses the influence of NH_3, L-glutamate, and other nitrogen sources on the activities of NAD- and NADP-GDH during the development of *S. commune* through basidiospore germination, homokaryotic mycelium growth, and dikaryotization.

MATERIALS AND METHODS

Culture conditions and strains of S. commune. Homokaryotic mycelium strains of *S. commune*, 699 A41B41, 845 A51B51, M1478 A41B41 lysine$^-$, 70 A2B1 uracil$^-$, and 667 A2B2 arginine$^-$, were cultured and mated on appropriately supplemented minimal medium, and basidiospores were collected as described previously (13). A homokaryotic fruiting strain, 35hf A41B51 (Mishkin and Niederpruem, *unpublished data*), was used to compare spores with the routinely used dikaryotic fruit progeny. Initial

http://www.pubmedcentral.nih.gov/picrender.fcgi?artid=276534&action=stream&blobtyp... 17/09/2004

JOURNAL OF BACTERIOLOGY, Jan. 1968, p. 87-94
Copyright © 1968 American Society for Microbiology
Vol. 95, No. 1
Printed in U.S.A.

Evidence for Two Species of Glutamate Dehydrogenases in *Thiobacillus novellus*

H. B. LÉJOHN AND BARBARA-ELLEN McCREA[1]
Department of Microbiology, University of Manitoba, Winnipeg 19, Manitoba, Canada

Received for publication 24 August 1967

When grown autotrophically in a thiosulfate-mineral salts medium, cells of the facultative chemoautotrophic bacterium, *Thiobacillus novellus*, produced two distinct glutamate dehydrogenases, one specific for nicotinamide adenine dinucleotide phosphate (NADP) and the other specific for nicotinamide adenine dinucleotide (NAD). When glutamate was supplied exogenously as the sole carbon source, the NAD-specific glutamate dehydrogenase was fully induced. Lower levels of the enzyme were found in bacteria grown in L-arginine, L-alanine, glucose, glycerol, lactate, citrate, or succinate. Arginine, histidine, and aspartate, on the other hand, caused a marked repression of the NADP-specific glutamate dehydrogenase activity. The NAD-dependent glutamate dehydrogenase was allosteric. Adenosine-5'-monophosphate and adenosine-5'-diphosphate acted as positive effectors. Both glutamate dehydrogenases were purified about 250-fold and were shown to be distinct protein with different physical properties.

It has been known for some time that the facultative chemoautotroph, *Thiobacillus novellus*, can grow rapidly on glutamate as a heterotrophic organism (10). In addition, *T. novellus* can be cultured as an autotroph in a medium composed entirely of mineral salts, when thiosulfate or some other reduced inorganic sulfur compound supplies energy and CO_2 is fixed via the Calvin-Benson pathway. In addition, glutamate can serve as a nitrogen and carbon source in a nitrogen-free medium (LéJohn, *unpublished data*). It is reasonable to assume that *T. novellus* possesses an active glutamate dehydrogenase [L-glutamate-NAD(P) oxidoreductase EC 1.4.1.3] that can catalyze the reversible breakdown of glutamate to provide the necessary carbon and nitrogen required for growth in glutamate. It seems logical then to expect that this enzyme, which is at an important cross-link of carbohydrate and nitrogen metabolism, is regulated in some way. Either there are two enzymes, each acting independently, favoring synthesis or breakdown or, alternatively, the same enzyme is under some form of regulation.

Glutamate dehydrogenases (GDH) have been widely studied in plants, animals, and fungi. A detailed review of this appeared recently (3). There is, as yet, relatively little information available on the GDH of bacteria. A widespread belief is that bacteria possess only one type of GDH, which is either specific for nicotinamide adenine dinucleotide (NAD) or for nicotinamide adenine dinucleotide phosphate (NADP). This report clearly shows that, in *T. novellus*, there are two distinct GDH, one specific for NAD and the other specific for NADP. The NAD-linked enzyme is inducible and allosteric (6).

[1] Predoctoral Fellow.

MATERIALS AND METHODS

Bacteria and culture conditions. A strain of *T. novellus* (ATCC 8093) was grown and harvested as described elsewhere (7). Bacterial cells were cultured in a glutamate-thiosulfate mineral salts medium to isolate the two GDH. During induction studies, the pertinent carbon source was substituted for glutamate.

Protein determination. Protein content was determined spectrophotometrically (11) and by the phenol method of Lowry et al. (8).

Growth. Growth was determined by measuring the optical density (OD) of the cells at 530 mμ by use of a Klett-Summerson photoelectric colorimeter (the cells were first filtered through loosely packed cotton plugs to remove colloidal sulfur that is deposited during growth).

Preparation of cell-free extracts. The pellet of harvested bacteria was resuspended as a 25 to 30% (wet weight/volume) suspension of cells in 0.05 M tris-(hydroxymethyl)aminomethane chloride (Tris chloride), *p*H 8, containing 10^{-4} M reduced glutathione (GSH) and 10^{-4} M adenosine-5'-monophosphate (AMP) (designated TGA-buffer). The cells were disrupted in a 10-kc Raytheon sonic disintegrator (Raytheon Co., South Norwalk, Conn.) for 30 min at 5 C. Cell debris was removed by centrifugation at 48,000 × *g* for 15 min at 2 C. Occasionally, particulate reduced

http://www.pubmedcentral.nih.gov/picrender.fcgi?artid=251975&action=stream&blobtyp... 17/09/2004

EMBL-EBI European Bioinformatics Institute
Get Nucleotide sequences for Go ? Site search
Site Map SPS
EBI Home About EBI Research Services Toolbox Databases Downloads
InterPro
InterPro home Text Search Sequence Search Databases Documentation FTP site Protein of the month
Search: Search Entries Search InterPro

InterPro **Glutamate synthase, amidotransferase region**

[?] = help

IPR006981
Glu_synth_NTN

Matches: 129 proteins. View matches:

Please be aware that match views for entries matching more than 1000 proteins may be slow.

Overview: sorted by AC, sorted by name, of known structure, grouped by taxonomy
Detailed: sorted by AC, sorted by name, of known structure
Table: For all matching proteins, of known structure
Architectures

Name [?] Glutamate synthase, amidotransferase region

Signatures [?] PF04897;Glu_synth_NTN (129 proteins)

Type [?] Domain

Dates [?] 2002-11-14 13:17:55.0 (created)
2002-11-14 13:17:55.0 (modified)

Process [?] nitrogen metabolism (GO:0006807)

Function [?] glutamate synthase activity (GO:0015930)

Abstract [?]

Glutamate synthase (GltS)1 is a key enzyme in the early stages of the assimilation of ammonia in bacteria, yeasts, and plants. In bacteria, L-glutamate is involved in osmoregulation, is the precursor for other amino acids, and can be the precursor for heme biosynthesis. In plants, GltS is especially essential in the reassimilation of ammonia released by photorespiration. On the basis of the amino acid sequence and the nature of the electron donor, three different classes of GltS can de defined as follows: 1) ferredoxin-dependent GltS (Fd-GltS), 2) NADPH-dependent GltS (NADPH-GltS), and 3) NADH-dependent GltS (properties of the three classes have been reviewed extensively [1]). The enzyme is a complex iron-sulfur flavoprotein catalyzing the reductive transfer of the amido nitrogen from L-glutamine to 2-oxoglutarate to form two molecules of L-glutamate via intramolecular channeling of ammonia from the amidotransferase domain to the FMN-binding domain.

Reaction of amidotransferase domain:

L-glutamine + H2O = L-glutamate + NH3

Reactions of FMN-binding domain:

2-oxoglutarate + NH3 = 2-iminoglutarate + H2O

2e + FMNox = FMNred

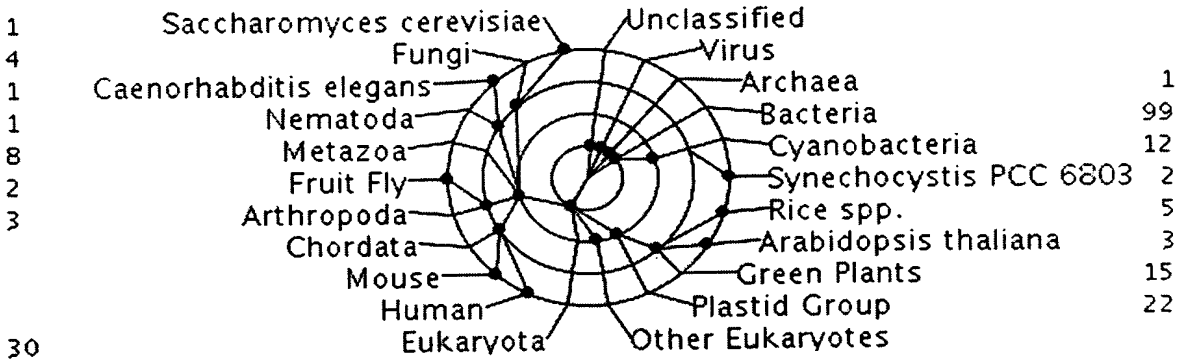
2-iminoglutarate + FMNred = L-glutamate + FMNox

The amidotransferase domain from Fd-GltS contains the typical catalytic center of N-terminal nucleophile amidotransferases. The overall topology is characterized by a four layer alpha/beta/beta/alpha architecture, which is similar to other N-terminal nucleophile amidotransferases [2].

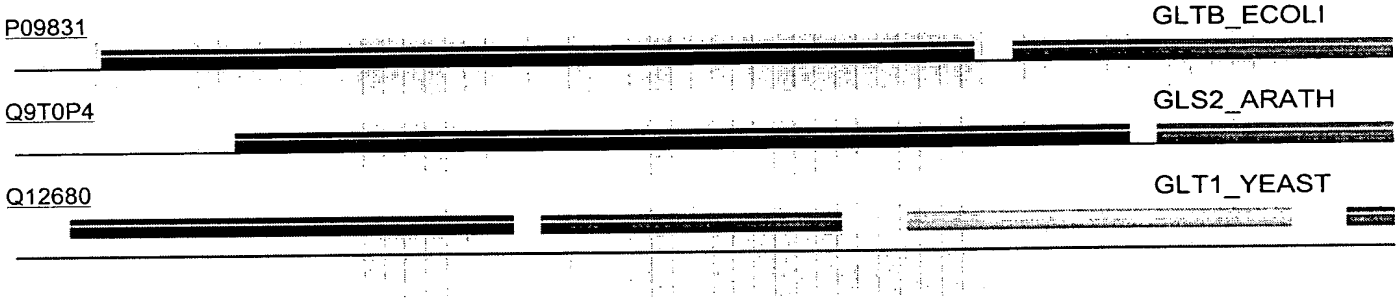
Structural links [?] PDB 1ea0, 1llw, 1llz, 1lm1
SCOP d.153.1.1

Database links [?] Enzyme 1.4.-.-

Taxonomy [?]



Examples



More proteins...

IPR006981 Glutamate synthase, amidotransferase region
IPR000103 Pyridine nucleotide-disulphide oxidoreductase, class-II
IPR000759 Adrenodoxin reductase
IPR001327 FAD-dependent pyridine nucleotide-disulphide oxidoreductase
IPR002489 Glutamate synthase, alpha subunit, C-terminal
IPR002932 Ferredoxin-dependent glutamate synthase
IPR003009 FMN/related compound-binding core
IPR006005 Glutamate synthase, NADH/NADPH, small subunit 1
IPR006982 Glutamate synthase, central

Publications

1. Vanoni M.A. , Curti B.
Glutamate synthase: a complex iron-sulfur flavoprotein.
Cell. Mol. Life Sci. 55: 617- 638 (1999) [PubMed: 10357231]

2. Van den heuvel R.H. , Ferrari D. , Bossi R.T. , Ravasio S. , Curti B. , Vanoni M.A. , Florencio F.J. , Mattevi A.
Structural studies on the synchronization of catalytic centers in glutamate synthase.
J. Biol. Chem. 277: 24579- 24583 (2002) [PubMed: 11967268]

Normal Printer Friendly Text Simple HTML XML Curator View

Page Maintained by EBI Support.

American Society of Plant Biologists | American Society of Plant Biologists | Amer Biolo

Membership | Meetings | Publications | Education | Job Bank | Public Affairs | Resource Links | Discussion Forums | ASPB HC

Expression of NADH-dependent glutamate synthase protein in epi- and exoderm roots in response to the supply of nitrogen

Abstract Number:**910**

Program Information

Session Number	Session Name
49	Interactions of C&N Metabolism

Presented by: Yamaya, Tomoyuki, tyamaya@biochem.tohoku.ac.jp

Authors:

Ishiyama, Keiki Tohoku University
Hirose, Naoya Tohoku University
Hayakawa, Toshihiko Tohoku University
Yamaya, Tomoyuki Tohoku University

The mRNA and protein for NADH-dependent glutamate synthase (NADH-GOGAT; EC 1.4.
root tips of rice (*Oryza sativa* L. cv Sasanishiki) seedlings, grown for 26 d in water alone, ac
markedly within 12 h of the start of a supply of a low concentration (>0.05 mM) of ammoniu
(PCP 36: 1197, 1995). This rapid response was analyzed by localizing the enzyme protein i
tissues and by accumulation of its mRNA. When the root tips (> 1 mm) were immuno-staine
affinity-purified anti-NADH-GOGAT IgG, the faint signals were mainly detected in the centra
Signals for Fd-GOGAT (EC 1.4.7.1) were also observed in the same area. In contrast, the r
which were supplied with 1 mM ammonium ions for 24 h showed strong signals for NADH-C
protein in two layers of the root surface, epidermis and exodermis, in addition to the central
The supply of the ions had no effect on the profile on signals for Fd-GOGAT. Cytosolic GS
located in those two cell layers, though the supply of ammonium ions had less effect on its
localization. The results suggest that NADH-GOGAT is the limiting step for the assimilation
ammonium ions, i.e. to provide Glu for the GS reaction and that the ions absorbed are assir
those two-layers of cells. The mRNA for NADH-GOGAT in the whole roots accumulated ma
within 6 h of the supply of the ions, then declined. CHI had no inhibitory effect on the accum
When MSX was added together with the ammonium ions, the accumulation of the mRNA w
detected, suggesting ammonium ion itself is not a direct signals for the expression of NADH
gene in rice roots. This work was supported by a program of Research for the Future from J
(JSPS-RFTF96L00604).

Technical comments? Email webmaster@abstracts.aspb.org
(**Note:** Please do not direct science questions to the webmaster. Direct them instead to the author/presenter of the particular abstract in question. ***Thank you!***)

NCBI PubMed National Library of Medicine NLM

Entrez PubMed Nucleotide Protein Genome Structure OMIM PMC Journals Boo

Search PubMed for Go Clear

Limits Preview/Index History Clipboard Details

Display Abstract Show: 20 Sort Send to Text

About Entrez

Text Version

Entrez PubMed
Overview
Help | FAQ
Tutorial
New/Noteworthy
E-Utilities

PubMed Services
Journals Database
MeSH Database
Single Citation Matcher
Batch Citation Matcher
Clinical Queries
LinkOut
Cubby

Related Resources
Order Documents
NLM Catalog
NLM Gateway
TOXNET
Consumer Health
Clinical Alerts
ClinicalTrials.gov
PubMed Central

1: Cell Mol Life Sci. 1999 Apr;55(4):617-38.

Related Articles

Glutamate synthase: a complex iron-sulfur flavoprotein.

Vanoni MA, Curti B.

Dipartimento di Fisiologia e Biochimica Generali, Universita degli Studi di Milano, Italy.

Glutamate synthase is a complex iron-sulfur flavoprotein that forms L-glutan from L-glutamine and 2-oxoglutarate. It participates with glutamine syntheta ammonia assimilation processes. The known structural and biochemical prop of glutamate synthase from Azospirillum brasilense, a nitrogen-fixing bacteri will be discussed in comparison to those of the ferredoxin-dependent enzyme from photosynthetic tissues and of the eukaryotic reduced pyridine nucleotid dependent form of glutamate synthase in order to gain insight into the mecha of the glutamate synthase reaction. Sequence analyses also revealed that the subunit of bacterial glutamate synthase may be the prototype of a novel class flavin adenine dinucleotide- and iron-sulfur-containing oxidoreductase widel used as an enzyme subunit or domain to transfer reducing equivalents from N (P)H to an acceptor protein or protein domain.

Publication Types:
- Review
- Review, Tutorial

PMID: 10357231 [PubMed - indexed for MEDLINE]

Display Abstract Show: 20 Sort Send to Text

Write to the Help Desk
NCBI | NLM | NIH
Department of Health & Human Services
Privacy Statement | Freedom of Information Act | Disclaimer

Sep 14 2004 18:26:21

PURDUE UNIVERSITY

Department of Horticulture and Landscape Arcl

HORT640 - Metabolic Plant Physiology

Ammonia Assimilation and Recycling

Glutamate Dehydrogenase

In certain fungi the main pathway of ammonia assimilation into glutamate involv NADPH-dependent glutamate dehydrogenase (GDH) [EC 1.4.1.4] (Sims and Fc 1951):

NH_3 + 2-oxoglutarate + NADPH + H^+ <---> glutamate + N

Direct ammonia assimilation into glutamate via the catalytic action of NADPH-G the food yeast *Candida utilis* by $^{15}NH_3$ tracer experiments and quantitative analy (Sims and Folkes, 1964).

In the filamentous fungus *Neurospora crassa* NADPH-GDH is encoded by the a 1980). In *Aspergillus nidulans* the NADPH-GDH is designated *gdhA* (Kinghorn a et al, 1986; Hawkins et al, 1989), while in *Saccharomyces cerevisiae* this gene i (Moye et al, 1985). These NADPH-GDHs are hexamers with subunit molecular (Britton et al, 1992).

In fungi and yeasts a second NADH-dependent GDH [EC 1.4.1.2] functions prin catabolism (Sanwal and Lata, 1961):

glutamate + NAD^+ <---> 2-oxoglutarate + NH_3 + NADH

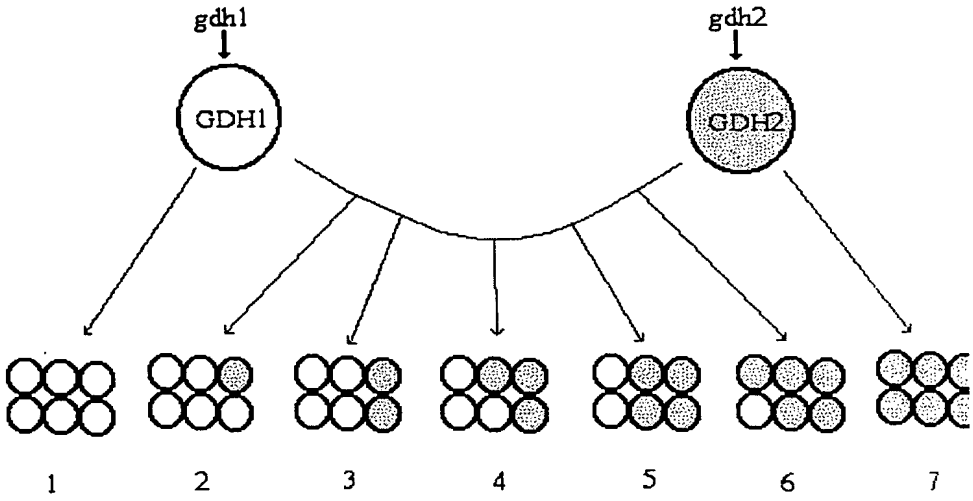
The NADH-GDH of the filamentous fungus *Neurospora crassa* is encoded by th 1993). The equivalent enzyme in *Aspergillus nidulans* is encoded by *gdhB* (King 1976). The NADH-GDHs of fungi and yeasts appear to be tetramers with much kDa) than the hexameric NADPH-GDHs involved in ammonia assimilation (Britt NADH-GDH of *Candida utilis* and *Saccharomyces cerevisiae* is regulated by rev involving phosphorylation/dephosphorylation of the enzyme (Hemmings and Sir 1978; 1981; Uno et al, 1984). The NADH-GDH of *Neurospora crassa* is regulate (Kapoor et al, 1993).

Most bacteria (and cyanobacteria) possess a single NADPH-GDH isoform of the *Corynebacterium glutamicum* [*gdh*] (Bormann et al, 1992); *Escherichia coli* [*gdh* Wootton, 1983); *Salmonella typhimurium* [*gdhA*] (Miller and Brenchley, 1984); a 6803 [*gdhA*] (Chavez et al, 1995). Like the NADPH-GDHs of fungi and yeasts, t GDHs are thought to be primarily involved in ammonia assimilation rather than However, *Bacteroides fragilis* Bf1 has two GDH activities. One is dual cofactor N (encoded by *gdhA*), while the other has NADH-specific activity (encoded by *gdh* 1998). The dual coenzyme-specific GDH is regulated by reversible inactivation ammonium in *Bacteroides fragilis* (Yamamoto et al, 1987). In contrast to yeast, reversible inactivation of *Bacteroides fragilis* NAD(P)H does not appear to involv (Yamamoto et al, 1987). The dual specific NAD(P)H-GDH of *Bacteroides thetai gdhA* is typical of the family I-type hexameric GDH proteins (Baggio and Morris bacterium *Psychrobacter* sp. TAD1 also contains two distinct glutamate dehydr specific for either NADPH or NADH (Di Fraia et al, 2000). The NADPH-GDH of hexameric structure (Di Fraia et al, 2000).

For missing text on right margin, see attachment

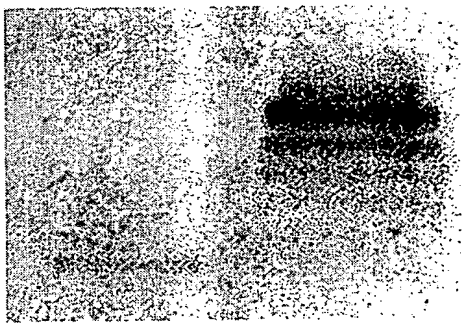
Two distinct genes (designated *GDH1* and *GDH2*) encoding NADH-GDH have l
Arabidopsis thaliana (Turano et al, 1997). Both gene products contain putative r
polypeptides and NADH- and 2-oxoglutarate-binding domains (Turano et al, 19
fractionation confirms the mitochondrial location of the NADH-GDH isoenzymes
GDH1 encodes a 43.0 kDa polypeptide, designated alpha, and *GDH2* encodes
designated beta (Turano et al, 1997). The two subunits combine in different rati
GDH isoenzymes (Turano et al, 1997). In *Arabidopsis*, the slowest-migrating isc
GDH1, is a homohexamer composed of alpha subunits, and the fastest-migratir
homohexamer composed of beta subunits (Turano et al, 1997). GDH isoenzym
heterohexamers composed of different ratios of alpha and beta subunits (Turan

Zea mays also possesses two distinct loci encoding NADH-GDH (Pryor, 1990).
Arabidopsis model described above (Turano et al, 1997), maize mutants deficie
show only a single GDH isozyme corresponding to isozyme 7 [i.e. the homohex
gdh2 gene product] (Magalhaes et al, 1990). The wildtype shows seven isozym



Isozymes of glutamate dehydrogenase (GDH) in plants

$$\text{glutamate} + NAD^+ + H_2O \underset{\text{GDH}}{\rightleftharpoons} \text{2-oxoglutarate} + NH_3 + NADH + H$$



**Native gel of GDH isozymes of the maize *gdh1*-null mutant (left) and wildt
were subjected to gel electrophoresis and stained for GDH as described b**

(see also discussion of GDH under gamma-aminobutyrate (GABA) metabolisn
reactions section)

In contrast to higher plants *Chlorella sorokiniana* has seven ammonium-inducibl GDH isozymes composed of varying ratios of alpha- and beta-subunits (Miller e *sorokiniana* genome possesses a single 7178 bp nuclear NADPH-GDH gene (N single gene produces two 2074 and 2116 nucleotide mRNAs encoding precursc 57,850 Da, respectively (Miller et al, 1998). The two NADPH-GDH mRNAs are i of a 42 nucleotide sequence located within the 5'-coding region of the longer mf This 42 nucleotide appears to undergo alternative splicing from the precursor m regulated by both nutritional and environmental signals (Miller et al, 1998). The subunits of 53,501 and 52,342 Da are identical in sequence except for an 11 an N-terminus of the alpha-subunit (Miller et al, 1998).

References

Abrahams GL, Abratt VR 1998 The NADH-dependent glutamate dehydrogenas *fragilis* Bf1 is induced by peptides in the growth medium. Microbiology 144: 165

Baggio L, Morrison M 1996 The NAD(P)H-utilizing glutamate dehydrogenase of *thetaiotaomicron* belongs to enzyme family I, and its activity is affected by *trans* downstream of *gdhA*. J. Bacteriol. 178: 7212-7220.

Britton KL, Baker PJ, Rice DW, Stillman TJ 1992 Structural relationship betwee tetrameric family of glutamate dehydrogenases. Eur. J. Biochem. 209: 851-859.

Chavez S, Reyes JC, Chauvat F, Florencio FJ, Candau P 1995 The NADP-glut the cyanobacterium *Synechocystis* 6803: cloning, transcriptional analysis and d gene. Plant Mol. Biol. 28: 173-188.

Di Fraia R, Wilquet V, Ciardiello MA, Carratore V, Antignani A, Camardella L, G 2000 $NADP^+$-dependent glutamate dehydrogenase in the Antarctic psychrotoler *Psychrobacter* sp. TAD1. Characterization, protein and DNA sequence, and rel glutamate dehydrogenases. Eur. J. Biochem. 267: 121-131.

Fincham JRS 1951 The occurrence of glutamate dehydrogenase in *Neurospora* in certain mutant strains. J. Gen. Microbiol. 5: 793-806.

Gurr SJ, Hawkins AR, Drainas C, Kinghorn JR 1986 Isolation and identification *gdhA* gene encoding NADP-linked glutamate dehydrogenase. Curr. Genet. 10:

Hawkins AR, Gurr SJ, Montague P, Kinghorn JR 1989 Nucleotide sequence an of the *Aspergillus nidulans gdhA* gene encoding NADP dependent glutamate de Genet. 218: 105-111.

Hemmings BA 1978 Phosphorylation of NAD-dependent glutamate dehydrogen Chem. 253: 5255-5258.

Hemmings BA 1981 Reactivation of the phospho form of the NAD-dependent gl by a yeast protein phosphatase. Eur. J. Biochem. 116: 47-50.

Hemmings BA, Sims AP 1977 The regulation of glutamate metabolism in *Candi* interconvertible forms of NAD-dependent glutamate dehydrogenase. Eur. J. Bio

Kapoor M, Vijayaraghavan Y, Kadonaga R, LaRue KE 1993 NAD(+)-specific gl *Neurospora crassa*: cloning, complete nucleotide sequence, and gene mapping 205-219.

Kinghorn JR, Pateman JA 1975 The structural gene for NADP L-glutamate deh

nidulans. J. Gen. Microbiol. 86: 294-300.

Kinsey JA, Fincham JR, Siddig MA, Keighren M 1980 New mutational variants c specific glutamate dehydrogenase. Genetics 95: 305-316.

Magalhaes JR, Ju GC, Rich PJ, Rhodes D 1990 Kinetics of $^{15}NH_4^+$ assimilation studies with a glutamate dehydrogenase (GDH1) null mutant. Plant Physiol. 94:

McPherson MJ, Wootton JC 1983 Complete nucleotide sequence of the *Escher* Nucleic Acids Res. 11: 5257-5266.

Miller ES, Brenchley JE 1984 Cloning and characterization of *gdhA*, the structur dehydrogenase of *Salmonella typhimurium*. J. Bacteriol. 157: 171-178.

Miller PW, Dunn WI, Schmidt RR 1998 Alternative splicing of a precursor-mRN/ *sorokiniana* NADP-specific glutamate dehydrogenase gene yields mRNAs for p isozyme subunits with different ammonium affinities. Plant Mol. Biol. 37: 243-63

Moye WS, Amuro N, Rao JK, Zalkin H 1985 Nucleotide sequence of yeast *GDH* adenine dinucleotide phosphate-dependent glutamate dehydrogenase. J. Biol. (

Pryor A 1990 A maize glutamate dehydrogenase null mutant is cold temperatur 367-372.

Sanwal BD, Lata M 1961 Glutamate dehydrogenase in single gene mutants of *I* amination. Nature (Lond.) 190: 286-287.

Sims AP, Folkes BF 1964 A kinetic study of the assimilation of ^{15}N-ammonia an acids in an exponentially growing culture of *Candida utilis*. Proc. Roy. Soc. Lonc 502.

Turano FJ, Thakkar SS, Fang T, Weisemann JM 1997 Characterization and exp dependent glutamate dehydrogenase genes in *Arabidopsis*. Plant Physiol. 113:

Uno I, Matsumoto K, Adachi K, Ishikawa T 1984 Regulation of NAD-dependent by protein kinases in *Saccharomyces cerevisiae*. J. Biol. Chem. 259: 1288-129:

Yamamoto I, Saito H, Ishimoto M 1987 Regulation of synthesis and reversible ir coenzyme-specific glutamate dehydrogenase in *Bacteroides fragilis*. J. Gen. Mi

| *Arabidopsis thaliana* Pathways (TAIR) | PubMed Search | Entrez Protein Search | ISI Web of Know

Google™ Google
○ www ◉ www.hort.purdue.edu

David Rhodes
Department of Horticulture & Landscape Architecture
Horticulture Building
625 Agriculture Mall Drive
Purdue University
West Lafayette, IN 47907-2010
Last Update: 8/16/2004

Copy of
http://www.hort.purdue.edu/rhodcv/hort640c/ammonig/am00001.htm

Department of Horticulture and Landscape Architecture

Ammonia Assimilation and Recycling

Glutamate Dehydrogenase
In certain fungi the main pathway of ammonia assimilation into glutamate involves the catalytic activity of NADPH-dependent glutamate dehydrogenase (GDH) [EC 1.4.1.4] (Sims and Folkes, 1964; Fincham, 1951):

NH3 + 2-oxoglutarate + NADPH + H+ <---> glutamate + NADP+
Direct ammonia assimilation into glutamate via the catalytic action of NADPH-GDH was demonstrated in the food yeast Candida utilis by 15NH3 tracer experiments and quantitative analysis of metabolic fluxes (Sims and Folkes, 1964).

In the filamentous fungus Neurospora crassa NADPH-GDH is encoded by the am gene (Kinsey et al, 1980). In Aspergillus nidulans the NADPH-GDH is designated gdhA (Kinghorn and Pateman, 1975; Gurr et al, 1986; Hawkins et al, 1989), while in Saccharomyces cerevisiae this gene is designated GDH1 (Moye et al, 1985). These NADPH-GDHs are hexamers with subunit molecular weights of ~ 50 kDa (Britton et al, 1992).

In fungi and yeasts a second NADH-dependent GDH [EC 1.4.1.2] functions primarily in glutamate catabolism (Sanwal and Lata, 1961):

glutamate + NAD+ <---> 2-oxoglutarate + NH3 + NADH + H+
The NADH-GDH of the filamentous fungus Neurospora crassa is encoded by the gene gdh (Kapoor et al, 1993). The equivalent enzyme in Aspergillus nidulans is encoded by gdhB (Kinghorn and Pateman, 1976). The NADH-GDHs of fungi and yeasts appear to be tetramers with much larger subunit size (~ 115 kDa) than the hexameric NADPH-GDHs involved in ammonia assimilation (Britton et al, 1992). The NADH-GDH of Candida utilis and Saccharomyces cerevisiae is regulated by reversible deactivation involving phosphorylation/dephosphorylation of the enzyme (Hemmings and Sims, 1977; Hemmings, 1978; 1981; Uno et al, 1984). The NADH-GDH of Neurospora crassa is regulated by catabolite repression (Kapoor et al, 1993).

Most bacteria (and cyanobacteria) possess a single NADPH-GDH isoform of the hexameric type: see e.g. Corynebacterium glutamicum [gdh] (Bormann et al, 1992); Escherichia coli [gdhA] (McPherson and Wootton, 1983); Salmonella typhimurium [gdhA] (Miller and Brenchley, 1984); and Synechocystis PCC 6803 [gdhA] (Chavez et al, 1995). Like the NADPH-GDHs of fungi and yeasts, these bacterial NADPH-GDHs are thought to be primarily involved in ammonia assimilation rather than glutamate catabolism. However, Bacteroides fragilis Bf1 has two GDH activities. One is dual

cofactor NAD(P)H-dependent (encoded by gdhA), while the other has NADH-specific activity (encoded by gdhB) (Abrahams and Abratt, 1998). The dual coenzyme-specific GDH is regulated by reversible inactivation in vivo and repression by ammonium in Bacteroides fragilis (Yamamoto et al, 1987). In contrast to yeast, the mechanism of reversible inactivation of Bacteroides fragilis NAD(P)H does not appear to involve phosphorylation (Yamamoto et al, 1987). The dual specific NAD(P)H-GDH of Bacteroides thetaiotaomicron encoded by gdhA is typical of the family I-type hexameric GDH proteins (Baggio and Morrison, 1996). The Antarctic bacterium Psychrobacter sp. TAD1 also contains two distinct glutamate dehydrogenases (GDH), each specific for either NADPH or NADH (Di Fraia et al, 2000). The NADPH-GDH of Psychrobacter has a hexameric structure (Di Fraia et al, 2000).

Two distinct genes (designated GDH1 and GDH2) encoding NADH-GDH have been identified in Arabidopsis thaliana (Turano et al, 1997). Both gene products contain putative mitochondrial transit polypeptides and NADH- and 2-oxoglutarate-binding domains (Turano et al, 1997). Subcellular fractionation confirms the mitochondrial location of the NADH-GDH isoenzymes (Turano et al, 1997). GDH1 encodes a 43.0 kDa polypeptide, designated alpha, and GDH2 encodes a 42.5 kDa polypeptide, designated beta (Turano et al, 1997). The two subunits combine in different ratios to form seven NADH-GDH isoenzymes (Turano et al, 1997). In Arabidopsis, the slowest-migrating isoenzyme in a native gel, GDH1, is a homohexamer composed of alpha subunits, and the fastest-migrating isoenzyme, GDH7, is a homohexamer composed of beta subunits (Turano et al, 1997). GDH isoenzymes 2 through 6 are heterohexamers composed of different ratios of alpha and beta subunits (Turano et al, 1997).

Zea mays also possesses two distinct loci encoding NADH-GDH (Pryor, 1990). Consistent with the Arabidopsis model described above (Turano et al, 1997), maize mutants deficient in gdh1 (Pryor, 1990) show only a single GDH isozyme corresponding to isozyme 7 [i.e. the homohexamer derived from the gdh2 gene product] (Magalhaes et al, 1990). The wildtype shows seven isozymes:

Native gel of GDH isozymes of the maize gdh1-null mutant (left) and wildtype (right). Root extracts were subjected to gel electrophoresis and stained for GDH as described by Magalhaes et al (1990).

(see also discussion of GDH under gamma-aminobutyrate (GABA) metabolism in the Aminotransferase reactions section)
In contrast to higher plants Chlorella sorokiniana has seven ammonium-inducible, chloroplastic NADPH-GDH isozymes composed of varying ratios of alpha- and beta-subunits (Miller et al, 1998). The C. sorokiniana genome possesses a single 7178 bp nuclear NADPH-GDH gene (Miller et al, 1998). This single gene produces two 2074 and

2116 nucleotide mRNAs encoding precursor proteins of 56,350 and 57,850 Da, respectively (Miller et al, 1998). The two NADPH-GDH mRNAs are identical with the exception of a 42 nucleotide sequence located within the 5'-coding region of the longer mRNA (Miller et al, 1998). This 42 nucleotide appears to undergo alternative splicing from the precursor mRNA by a process that is regulated by both nutritional and environmental signals (Miller et al, 1998). The mature alpha- and beta-subunits of 53,501 and 52,342 Da are identical in sequence except for an 11 amino acid extension at the N-terminus of the alpha-subunit (Miller et al, 1998).

References

Abrahams GL, Abratt VR 1998 The NADH-dependent glutamate dehydrogenase enzyme of Bacteroides fragilis Bf1 is induced by peptides in the growth medium. Microbiology 144: 1659-1667.

Baggio L, Morrison M 1996 The NAD(P)H-utilizing glutamate dehydrogenase of Bacteroides thetaiotaomicron belongs to enzyme family I, and its activity is affected by trans-acting gene(s) positioned downstream of gdhA. J. Bacteriol. 178: 7212-7220.

Britton KL, Baker PJ, Rice DW, Stillman TJ 1992 Structural relationship between the hexameric and tetrameric family of glutamate dehydrogenases. Eur. J. Biochem. 209: 851-859.

Chavez S, Reyes JC, Chauvat F, Florencio FJ, Candau P 1995 The NADP-glutamate dehydrogenase of the cyanobacterium Synechocystis 6803: cloning, transcriptional analysis and disruption of the gdhA gene. Plant Mol. Biol. 28: 173-188.

Di Fraia R, Wilquet V, Ciardiello MA, Carratore V, Antignani A, Camardella L, Glansdorff N, Di Prisco G 2000 NADP+-dependent glutamate dehydrogenase in the Antarctic psychrotolerant bacterium Psychrobacter sp. TAD1. Characterization, protein and DNA sequence, and relationship to other glutamate dehydrogenases. Eur. J. Biochem. 267: 121-131.

Fincham JRS 1951 The occurrence of glutamate dehydrogenase in Neurospora and its apparent absence in certain mutant strains. J. Gen. Microbiol. 5: 793-806.

Gurr SJ, Hawkins AR, Drainas C, Kinghorn JR 1986 Isolation and identification of the Aspergillus nidulans gdhA gene encoding NADP-linked glutamate dehydrogenase. Curr. Genet. 10: 761-766.

Hawkins AR, Gurr SJ, Montague P, Kinghorn JR 1989 Nucleotide sequence and regulation of expression of the Aspergillus nidulans gdhA gene encoding NADP dependent glutamate dehydrogenase. Mol. Gen. Genet. 218: 105-111.

Hemmings BA 1978 Phosphorylation of NAD-dependent glutamate dehydrogenase from yeast. J. Biol. Chem. 253: 5255-5258.

Hemmings BA 1981 Reactivation of the phospho form of the NAD-dependent glutamate dehydrogenase by a yeast protein phosphatase. Eur. J. Biochem. 116: 47-50.

Hemmings BA, Sims AP 1977 The regulation of glutamate metabolism in Candida utilis. Evidence for two interconvertible forms of NAD-dependent glutamate dehydrogenase. Eur. J. Biochem. 80: 143-151.

Kapoor M, Vijayaraghavan Y, Kadonaga R, LaRue KE 1993 NAD(+)-specific glutamate dehydrogenase of Neurospora crassa: cloning, complete nucleotide sequence, and gene mapping. Biochem. Cell Biol. 71: 205-219.

Kinghorn JR, Pateman JA 1975 The structural gene for NADP L-glutamate dehydrogenase in Aspergillus nidulans. J. Gen. Microbiol. 86: 294-300.

Kinsey JA, Fincham JR, Siddig MA, Keighren M 1980 New mutational variants of Neurospora NADP-specific glutamate dehydrogenase. Genetics 95: 305-316.

Magalhaes JR, Ju GC, Rich PJ, Rhodes D 1990 Kinetics of 15NH4+ assimilation in Zea mays: Preliminary studies with a glutamate dehydrogenase (GDH1) null mutant. Plant Physiol. 94: 647-656.

McPherson MJ, Wootton JC 1983 Complete nucleotide sequence of the Escherichia coli gdhA gene. Nucleic Acids Res. 11: 5257-5266.

Miller ES, Brenchley JE 1984 Cloning and characterization of gdhA, the structural gene for glutamate dehydrogenase of Salmonella typhimurium. J. Bacteriol. 157: 171-178.

Miller PW, Dunn WI, Schmidt RR 1998 Alternative splicing of a precursor-mRNA encoded by the Chlorella sorokiniana NADP-specific glutamate dehydrogenase gene yields mRNAs for precursor proteins of isozyme subunits with different ammonium affinities. Plant Mol. Biol. 37: 243-63.

Moye WS, Amuro N, Rao JK, Zalkin H 1985 Nucleotide sequence of yeast GDH1 encoding nicotinamide adenine dinucleotide phosphate-dependent glutamate dehydrogenase. J. Biol. Chem. 260: 8502-8508.

Pryor A 1990 A maize glutamate dehydrogenase null mutant is cold temperature sensitive. Maydica 35: 367-372.

Sanwal BD, Lata M 1961 Glutamate dehydrogenase in single gene mutants of Neurospora deficient in amination. Nature (Lond.) 190: 286-287.

Sims AP, Folkes BF 1964 A kinetic study of the assimilation of 15N-ammonia and the synthesis of amino acids in an exponentially growing culture of Candida utilis. Proc. Roy. Soc. Lond. B. Biol. Sci. 159: 479-502.

Turano FJ, Thakkar SS, Fang T, Weisemann JM 1997 Characterization and expression of NAD(H)-dependent glutamate dehydrogenase genes in Arabidopsis. Plant Physiol. 113: 1329-1341.

Uno I, Matsumoto K, Adachi K, Ishikawa T 1984 Regulation of NAD-dependent glutamate dehydrogenase by protein kinases in Saccharomyces cerevisiae. J. Biol. Chem. 259: 1288-1293.

Yamamoto I, Saito H, Ishimoto M 1987 Regulation of synthesis and reversible inactivation in vivo of dual coenzyme-specific glutamate dehydrogenase in Bacteroides fragilis. J. Gen. Microbiol. 133: 2773-2780.

| Arabidopsis thaliana Pathways (TAIR) | PubMed Search | Entrez Protein Search | ISI Web of Knowledge Search | Scirus Search |

PURDUE UNIVERSITY

Department of Horticulture and Landscape Architecture

HORT640 - Metabolic Plant Physiology

Ammonia Assimilation and Recycling

Regulation of glutamine synthetase

Fungi and Yeasts

- GS is encoded by the gene *GLN1* in *Saccharomyces cerevisiae* (Mitchell, 1985).
- GS is regulated by repression/derepression (mediated by glutamine). Mutants of the yeast *Saccharomyces cerevisiae* have been isolated which fail to derepress glutamine synthetase upon glutamine limitation. The mutations define a single nuclear gene, *GLN3* (Mitchell and Magasanik, 1984b). The elevated NAD-GDH activity normally found in glutamate-grown cells is not found in *gln3* mutants (Mitchell and Magasanik, 1984b). Glutamine limitation of *gln1* structural mutants has the opposite effect, causing elevated levels of NAD-GDH even in the presence of ammonia (Mitchell and Magasanik, 1984b). A regulatory circuit that responds to glutamine availability through the *GLN3* product has been proposed (Mitchell and Magasanik, 1984b).
- Mitchell and Magasanik (1984c) propose that production of GS in *Saccharomyces cerevisiae* is controlled by three regulatory systems. One system responds to glutamine levels and depends on the positively acting *GLN3* product (Mitchell and Magasanik, 1984c). The second system is general amino acid control, which couples derepression of a variety of biosynthetic enzymes to starvation for many single amino acids (Mitchell and Magasanik, 1984c). This system operates through the positive regulatory element *GCN4*. A third system responds to purine limitation (Mitchell and Magasanik, 1984c).
- It is now known that in *Saccharomyces cerevisiae*, the transcription factors Gln3p and Nil1p of the GATA family play a determinant role in expression of genes that are subject to nitrogen catabolite repression (Soussi-Boudekou and Andre, 1999). In addition, the yeast mutant, *gan1-1*, exhibits dramatically decreased NAD-GDH and GS activities (Soussi-Boudekou and Andre, 1999). The *GAN1* gene encodes a 488-amino-acid polypeptide bearing no typical DNA binding domain (Soussi-Boudekou and Andre, 1999). Gan1p is required for full expression of *GLN1*, *GDH2* and also other nitrogen utilization genes, including *GAP1*, *PUT4*, *MEP2* and *GDH1* (Soussi-Boudekou and Andre, 1999).
- In the food yeast *Candia utilis* GS is subject to cumulative feedback inhibition by end-products of glutamine metabolism *in vitro*, but this regulation was not demonstrable *in vivo* by direct measurements of the rate of glutamine synthesis (Sims and Ferguson, 1974).
- In *Candida utilis*, GS is regulated by glutamine-mediated repression and reversible deactivation involving dissociation of active octomers into deactive tetramers (Ferguson and Sims, 1971; 1974b; Sims et al, 1974a). Sims and coworkers demonstrated a rapid inactivation of GS in *Candida utilis* on the addition of ammonia to glutamate-grown cultures. An increase in glutamine and a decrease in 2-oxoglutarate is implicated in this control (cf. glutamine/2-oxoglutarate ratio involvement in control of GS adenylation/deadenylation in gram-negative bacteria) (Ferguson and Sims, 1971; 1974b; Sims et al, 1974a). High glutamine concentrations promote the "relaxation" of the native 15.4 S enzyme into a 14.2 S octamer which dissociates reversibly into two 8.7 S tetramers. PEP promotes relaxation and formation of enzyme tetramers.

NAD^+, NADPH and ATP cause dissociation of tetramers into monomers. Glutamate and Mg^{2+} prevent dissociation and promote reassociation of tetramers (Sims et al, 1974b). Whereas 2-oxoglutarate can prevent dissociation of octamers it cannot promote reassociation (Sims et al, 1974b). *Candida utilis* GS tetramers have the same transferase activity as octamers, but have reduced synthetase activity. In the presence of 2-oxoglutarate and glutamate the enzyme can maintain its structural integrity under conditions which would otherwise lead to dissociation (Sims et al, 1974b).

- In *Saccharomyces cerevisiae* GS is modulated by nitrogen repression and by two distinct inactivation processes (Legrain et al, 1982). Addition of glutamine to exponentially grown yeast leads to rapid enzyme inactivation that is reversed by removing glutamine from the growth medium (Legrain et al, 1982). A regulatory mutation (*gdhCR* mutation) suppresses this inactivation by glutamine in addition to its derepressing effect on enzymes involved in nitrogen catabolism (Legrain et al, 1982). The *gdhCR* mutation also increases the level of proteinase B in exponentially grown yeast (Legrain et al, 1982). Inactivation of GS is also observed during nitrogen starvation (Legrain et al, 1982). This inactivation is irreversible and consists very probably of a proteolytic degradation. Strains bearing proteinase A, B and C mutations are no longer inactivated under nitrogen starvation (Legrain et al, 1982).
- The reversible *in vivo* inactivation of *Saccharomyces cerevisiae* GS by the addition of glutamine or ammonia is characterized by a specific loss of synthetase activity; transferase activity remains stable (Mitchell and Magasanik, 1984b). Several physiological perturbations cause inactivation, such as carbon starvation or limitation for a required amino acid, which could cause a buildup of glutamine (Mitchell and Magasanik, 1984b). In contrast to *Candida utilis*, no change in the native size of the enzyme was associated with inactivation of *Saccharomyces cerevisiae* GS, but there appears to be a change in the immunological properties of the enzyme subunit (Mitchell and Magasanik, 1984b).
- *Neurospora crassa* GS is unusual in that it contains two non-identical polypeptides (Sanchez et al, 1980). When *Neurospora crassa* is grown exponentially on ammonium excess, ammonium is fixed by a glutamate dehydrogenase and an octameric glutamine synthetase (GS) (Lara et al, 1982). The synthesis of this GS polypeptide (beta) is regulated by the nitrogen source present in the medium; high on glutamate, intermediate on ammonium, and low on glutamine (Lara et al, 1982). However, when *N. crassa* is grown in fed-batch ammonium-limited cultures a different polypeptide of GS (alpha), arranged as a tetramer, is synthesized (Dunn-Coleman and Garrett, 1980; Lara et al, 1982). The tetrameric alpha GS is proposed to function with glutamate synthase in the assimilation of low ammonium concentrations (Lara et al, 1982). *gln-1b* mutant strains synthesize only the GS alpha monomer (i.e. lacks the GS beta monomer) (Calderon et al, 1990).

References

Calderon J, Martinez LM, Mora J 1990 Isolation and characterization of a *Neurospora crassa* mutant altered in the alpha polypeptide of glutamine synthetase. J. Bacteriol. 172: 4996-5000.

Dunn-Coleman NS, Garrett RH 1980 The role of glutamine synthetase and glutamine metabolism in nitrogen metabolite repression, a regulatory phenomenon in the lower eukaryote *Neurospora crassa*. Mol. Gen. Genet. 179: 25-32.

Ferguson AR, Sims AP 1971 Inactivation *in vivo* of glutamine synthetase and NAD-specific glutamate dehydrogenase: its role in the regulation of glutamine synthesis in yeasts. J. Gen. Microbiol. 69: 423-427.

Ferguson AR, Sims AP 1974a The regulation of glutamine metabolism in *Candida utilis*: the role of glutamine in the control of glutamine synthetase. J. Gen. Microbiol. 80: 159-171.

Ferguson AR, Sims AP 1974b The regulation of glutamine metabolism in *Candida utilis*: the inactivation of glutamine synthetase. J. Gen. Microbiol. 80: 173-185.

Lara M, Blanco L, Campomanes M, Calva E, Palacios R, Mora J 1982 Physiology of ammonium assimilation in *Neurospora crassa*. J. Bacteriol. 150: 105-112.

Legrain C, Vissers S, Dubois E, Legrain M, Wiame JM 1982 Regulation of glutamine synthetase from *Saccharomyces cerevisiae* by repression, inactivation and proteolysis. Eur. J. Biochem. 123: 611-616.

Mitchell AP 1985 The *GLN1* locus of *Saccharomyces cerevisiae* encodes glutamine synthetase. Genetics 111: 243-258.

Mitchell AP, Magasanik B 1984a Regulation of glutamine-repressible gene products by the *GLN3* function in *Saccharomyces cerevisiae*. Mol. Cell Biol. 4: 2758-2766.

Mitchell AP, Magasanik B 1984b Biochemical and physiological aspects of glutamine synthetase inactivation in *Saccharomyces cerevisiae*. J. Biol. Chem. 259: 12054-12062.

Mitchell AP, Magasanik B 1984c Three regulatory systems control production of glutamine synthetase in *Saccharomyces cerevisiae*. Mol. Cell Biol. 4: 2767-2773.

Sanchez F, Calva E, Campomanes M, Blanco L, Guzman J, Saborio JL, Palacios R 1980 Heterogeneity of glutamine synthetase polypeptides in *Neurospora crassa*. J. Biol. Chem. 255: 2231-2234.

Sims AP, Ferguson AR 1974 The regulation of glutamine metabolism in *Candida utilis*: studies with $^{15}NH_3$ to measure *in vivo* rates of glutamine synthesis. J. Gen. Microbiol. 80: 143-158.

Sims AP, Toone J, Box V 1974a The regulation of glutamine synthesis in the food yeast *Candida utilis*: the purification and subunit structure of glutamine synthetase and aspects of enzyme deactivation. J. Gen. Microbiol. 80: 485-499.

Sims AP, Toone J, Box V 1974b The regulation of glutamine metabolism in *Candida utilis*: mechanisms of control of glutamine synthetase. J. Gen. Microbiol. 84: 149-162.

Soussi-Boudekou S, Andre B 1999 A co-activator of nitrogen-regulated transcription in *Saccharomyces cerevisiae*. Mol. Microbiol. 31: 753-762.

| Glutamine synthetase (GS) (ExPASy) | *Arabidopsis thaliana* Pathways (TAIR) | PubMed Search | Entrez Protein Search | ISI Web of Knowledge Search | Scirus Search |

Google Search

PubMed Central
· Journal List
· Search
· Utilities

Plant Physiology
AMERICAN SOCIETY OF PLANT BIOLOGISTS

PubMed Central
■ Abstract
▸ Full Text
▸ PDF
▸ Contents
▸ Archive

PubMed
Articles by:
▸Trepp, G.
▸Plank, D.
▸Stephen Gantt, J.
▸Vance, C.

and links to:
Related articles
Show

Copyright © 1999, American Society of Plant Physiologists
Plant Physiol. 1999 March; 119 (3): 829–838

NADH-Glutamate Synthase in Alfalfa Root Nodules. Immunocytochemical Localization[1]

Gian B. Trepp, David W. Plank, J. Stephen Gantt, and Carroll P. Vance*

Institut für Pflanzenwissenschaften Eidgenössische Technische Hochschule-Zürich, 8092 Zurich, Switzerland (G.B.T.)

Department of Agronomy and Plant Genetics (G.B.T., D.W.P., C.P.V) and Department of Plant Biology (J.S.G.), University of Minnesota, St. Paul, Minnesota 55108

University of Minnesota, St. Paul, Minnesota 55108United States Department of Agriculture, Agricultural Research Service, Plant Science Research Unit, University of Minnesota, St. Paul, Minnesota 55108 (C.P.V.)

* Corresponding author; e-mail vance004@maroon.tc.umn.edu; fax 1–651–625–5058.

Received September 14, 1998; Accepted December 9, 1998.

This article has been cited by other articles in PMC.

ABSTRACT

In root nodules of alfalfa (*Medicago sativa* L.), N_2 is reduced to NH_4^+ in the bacteroid by the nitrogenase enzyme and then released into the plant cytosol. The NH_4^+ is then assimilated by the combined action of glutamine synthetase (EC 6.3.1.2) and NADH-dependent Glu synthase (NADH-GOGAT; EC 1.4.1.14) into glutamine and Glu. The alfalfa nodule NADH-GOGAT protein has a 101-amino acid presequence, but the subcellular location of the protein is unknown. Using immunocytochemical localization, we determined first that the NADH-GOGAT protein is found throughout the infected cell region of both 19- and 33-d-old nodules. Second, in alfalfa root nodules NADH-GOGAT is localized predominantly to the amyloplast of infected cells. This finding, together with earlier localization and fractionation studies, indicates that in alfalfa the infected cells are the main location for the initial assimilation of fixed N_2.

AJOL Home | About | Article order info. | Register | Search Supported by INASP

Western Indian Ocean Journal of Marine Science 180

Some Properties of Glutamate Dehydrogenase from the Marine Red Alga *Gracilaria sordida* (Harv.) W. Nelson

Request Article

Research Support Tool

For this peer-reviewed article

Capture Cite

View Metadata

Context

Author Bio

Other Works

Define Terms

Book Reviews

Dissertations

Online Forums

Quotations

Pay-Per-View

Media Reports

Google Search

Action

Email Author

Email Others

Matern SP Mtolera, *Institute of Marine Sciences, University of Dar es Salaam, P.O. Box 668, Zanzibar, Tanzania*

Abstract

Keywords: ammonia assimilation, glutamate dehydrogenase, GDH, *Gracilaria sordida*, red alga, enzyme activity

Glutamate dehydrogenases (GDH, EC 1.4.1.2-4) catalyse the entry of ammonium into the organic cycle via amination of a-ketoglutarate and its release via deamination of Lglutamate. NAD-GDH facilitates NAD-/NADP-dependent deamination while NADPH-GDH facilitates NADH-/NADPH-dependent amination reactions. In the study reported here, GDHs were extracted from the red alga *Gracilaria sordida* and purified 10- to 180-fold before examining their amination and deamination reaction properties. NAD-/NADP- and NADH-/ NADPH-dependent activities were the order of 11:1 and 1:1.8, respectively. The pH optima for amination and deamination were 8.2 and 8.4 under NADH- and NADPH- and 8.4 and 9 under NAD- and NADP- dependent activities, respectively. Whereas both NAD- and NADP-dependent deamination activities were activated by calcium ions (Ca^{2+}), only NADPH-dependent amination was activated. The K_m values (in mM) were 3.0–3.6 for ammonia, 2.0–3.3 for a-ketoglutarate, 0.00286 for NADH, 0.0033 for NADPH),1.7–2.1 for L-glutamate, 0.344 for NAD and 0.476 for NADP. It appears that the GDHs in *G. sordida* are dominated by NAD-dependent deamination enzyme and that in NADH-NADPH- dependent amination reactions, NADPH is more preferred. It is suggested that NAD-GDH's role in *G. sordida* could be as a catabolic shunt facilitating respiration. Anabolic functions could be assimilation of ammonia released during photorespiration and synthesis of N-rich transport compounds.

Western Indian Ocean Journal of Marine Sciences Vol.2(2) 2003: 179-186